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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of:				
Applicants	Leonard D. Kohn, et al.	:	Paper No:	79661/502168
Serial No.	09/151,612	:	Group Art Unit:	1633
Filed:	September 11, 1998	:	Examiner:	Q. Nguyen

For: IMMUNE ACTIVATION BY DOUBLE-STRANDED POLYNUCLEOTIDES

DECLARATION UNDER 37 CFR 1.132

Box Amendment Fee
The Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. This declaration under 37 CFR Sec. 1.132 is supportive of the Amendment filed herewith.

2. I, Leonard D. Kohn, have been employed by Ohio University since January 1, 2001, and that from June of 1994 to December 31, 2000, I was employed by the National Institutes of Health, and I was and still am, engaged in a research program in the field of immunology and particularly autoimmunity and related diseases.

3. I have reviewed the October 12, 2001, Office Action in the above captioned case and I am familiar with the references cited by the Examiner.

4. I disagree with the Examiner's position and maintain that one of ordinary skill in the field of immunology and autoimmunity would find that the present specification is enabled for use in providing enhanced immune recognition and in treating a variety of autoimmunity diseases. First, the Applicants' invention teaches a detailed description of how to elicit an *in vivo* immune response in a mouse model that adapts *in vitro* cell engineering and immune procedures described in multiple published reports [Shimojo, N., Kohn, Y., Yamaguchi, K-I., Kikuoka, S-I., Hoshioka, A., Niimi, H., Hirai, A., Tamura, Y., Saito, Y., Kohn, L. D., and Tahara, K. (1996). Proc. Natl. Acad. Sci. U.S.A. 93:11074-11079; Yamaguchi, K-I., Shimojo, N., Kikuoka, S., Hoshioka, A., Hirai, A., Tahara, K., Kohn, L. D., Kohno, Y., and Niimi, H. (1997) J. Clin. Endocrinol. Metab. 82:4266-4269; and Kikuoka, S., Shimojo, N., Yamaguchi, K-I., Watanabe, Y., Hoshioka, A., Hirai, A., Saito, Y., Tahara, K., Kohn, L.D., Kohno, Y., and Niimi, H. (1998) Endocrinology 139:1891-1989)]. The Shimojo model procedure has been repeated by the Davies group (Kita, M., Ahmad, L., Marians, R.C., Vlase, H., Unger, P., Graves', P.N., and Davies, T.F. (1999) Endocrinology 140:1392-1398. Further, using the same immunization procedure protocol plus thyroid peroxidase

(TPO), the Rapoport group generated autoantibodies to TPO that, unlike direct immunization procedures, for the first time mimicked the properties of autoantibodies to TPO in human subjects with autoimmune thyroiditis (Hashimoto's disease). [Jaume, J.C., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S.M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657]. In each case the immunization protocol, i.e. number of cells injected, method of injection (intraperatoneal), use of mitomycin treated cells, times of immunization, and conditions were effectively the same as used herein to develop a protective immune response. Further experimentation was not needed. Second, the Jaume et al report gave strong support that the procedure can be adapted to develop protective immune responses which require a comparable *in vivo* immune response in other animal models including humans, since for the first time a procedure generated TPO antibodies seen in human subjects. It was therefore likely we could show that the procedure could be adapted to develop a protective immune response. Finally, it is now recognized that a pathologic immune response starts as a protective response to eliminate damaged or viral infected cells. The disease is a pathologic overshoot of the initial protective response. The tumor model is a disease model that is protective. Applicants teach how to create an autoimmune disease killing the tumor or its cells akin to diabetes wherein the islet cells are destroyed. With normal islet cells this kills them and is a disease. With tumor cells the same result is protective.

5. I state we have done the following additional experimental work to support these contentions:

The 18R rat thyroid tumor was created by Wolman (J. Natl. Cancer Institute, 1961, 26: 676-685). It was one of a series of tumors developed in Fischer rats by chronic administration of thyrotropin (TSH) preparations. It was derived from a single tumor and carried by a subcutaneous implantation procedure (Wolman). Cells from the tumor were obtained by trypsinization and grown in the same medium used to grow FRTL-5 rat thyroid cells with the exception that the cells do not require the 6 hormone mixture for growth as do FRTL-5 cells, *i.e.*, growth is TSH independent.

Tumors were reestablished by injecting a pellet of cultured cells suspended in PBS into Fisher rats. When tumors were growing in 15 rats, they were further treated as follows. Five rats received injections of saline. Five received 10^7 mitomycin-treated cells that had been subjected to mock transfection with lipofectin; cells were in PBS and introduced by intraperitoneal injection. Five received 10^7 mitomycin-treated cells that had been transfected with ds polynucleotide, again in PBS and by intraperitoneal injection. In this experiment, we used polyI-C (Exp. 1). We repeated this experiment with poly dIdC (Exp. 2). The regimen was as described in the Shimojo paper to create Graves' Disease or in the patent wherein we created Graves' Disease with polynucleotide transfection in fibroblasts overexpressing TSHR, *i.e.*, injections every other week for 6 weeks. The oligonucleotide length was 35 bp. The transfected amount was 50 micrograms.

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Results below indicate significant reduction in tumor size:

Tumor Size after 16 weeks

Exp.1	PBS alone	Cells Alone subjected to Mock transfection	Cells Rxed with Polynucleotide
1	5.5 cm	5 cm	0.5 cm
2	5 cm	4.5 cm	0.5 cm
3	4cm	4 cm	<0.5 cm
4	3.5 cm	4 cm	<0.5 cm
5	4.5 cm	3.5 cm	0.5 cm
Exp.2			
1	3.5 cm	4.5 cm	<0.5 cm
2	3 cm	4 cm	<0.5 cm
3	4.5 cm	3.5 cm	1 cm
4	4 cm	4 cm	<0.5 cm
5	3.5 cm	3 cm	0.5 cm

6. I hereby declare that these results show that the present methods can be used *in vivo* to treat tumors and that the claims of the present application are enabled for one skilled in the art. Applicants' invention teaches a detailed description of how to create an immune response in vivo as measured by creation of an autoimmune disease mimicking Graves' disease in a mouse model. We show that that this procedure can be readily adapted to develops an *in vivo* protective immune response in a mouse system and therefore can be adapted to develop protective immune responses which require a comparable *in vivo* immune response in other animal models including humans. We suggest that adaption to humans is a reasonable supposition given the fact the Jaume mouse model which used the same procedure developed TPO antibodies which for the first time mimicked human antibodies, in contrast to previous immunization procedures.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Leonard D Kohn
Leonard Kohn

6/27/02
Date:

No. 09/151,612

Of Leonard D. Kohn , et a.

Title For IMMUNE ACTIVATION BY DOUBLE-STRANDED POLYNUCLEOTIDES

Action Amendment and Response; Declaration Under 37 CFR 1.132 Confirm te fax

SRA/RW 79661/502168 7/1/02

Please place the official stamp of the Patent Office on this card and return it to us for our files to constitute an acknowledgment by this Patent Office of receipt on the date stamped of the above identified paper.

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